Improving ecological fitness and environmental stress tolerance of the biocontrol yeast *Candida sake* by manipulation of intracellular sugar alcohol and sugar content

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Candida sake was cultured on nutrient yeast dextrose broth, which was diluted and/or modified by the addition of either glycerol, glucose to 0·96 or trehalose to 0·97 water activity (a_w) to modify endogenous sugar alcohol and sugar content. Sugar alcohols (glycerol, erythritol, arabitol and mannitol) and sugars (trehalose and glucose) were extracted from the yeast cells and quantified using HPLC. Total polyol and sugar content varied significantly between treatments. The total intracellular concentrations in NYDB medium were significantly increased in NYDB + glucose media. The major intracellular polyols/sugars in cells grown on unmodified NYDB were arabitol, trehalose and glucose with small amounts of glycerol and erythritol. This was changed by reducing a_w of the growth medium, particularly with glucose or glycerol. The major polyols in *C. sake* cells grown on glucose-modified media were arabitol and the low mol wt polyol glycerol, with smaller amounts of glucose. In glycerol-amended full strength normal and diluted media, glycerol was the major intracellular polyol with lower amounts of the other polyols and sugars. The viability of the yeast cells with modified polyols/sugars was significantly improved at lowered a_w level (0·935 and 0·95) when compared to unmodified yeast cells. Improvements in water stress tolerance was better in yeast cells grown for 48 than 24 h, especially in those from NYDB modified with either glucose, glycerol or trehalose. Such modifications of endogenous reserves were also shown to preserve or improve the biocontrol potential of the yeast against *Penicillium expansum* rot of apples. Thus physiological manipulation of intracellular polyol and sugar content may provide a means for developing biocontrol agents with improved ecological fitness in field environments.

Biological control using microbial antagonists has attracted much interest as an alternative to chemical methods of controlling pre- and post-harvest plant pathogens and pests of agricultural and horticultural crops (Janisiewicz, 1988; Wilson & Chalutz, 1989; Wilson & Wisniewski, 1989; Janisiewicz, 1990). Much research effort has been spent into optimizing spore yields and improvement of formulations of inocula but surprisingly little on improving physiological quality of inocula. Indeed, biocontrol in the field has often been severely limited by a narrow range of relative humidity and temperature conditions over which successful establishment and effective pest or disease control can occur (Doberski, 1981; Heale, 1988; Hallsworth & Magan, 1994*a*, *b*).

In recent years particular success has been achieved by the development of microbial antagonists effective against fungal pathogens of pome and citrus fruit, some of which are being commercially developed (Pusey & Wilson, 1984; Pusey *et al*., 1988; Janisiewicz & Marchi, 1992; Janisiewicz & Bors, 1995). Pre-harvest application in the field to such crops has not, however, been successful because of the environmental sensitivity of biocontrol strains (Wilson *et al*., 1991).

Recently, detailed studies have shown that a strain of *Candida sake* (K. Saito & M. Ota) N. van Uden & H. R. Buckley (CPA-1) is an effective antagonist to the major fungal pathogens of apples and pears (Usall, 1995; Viñas *et al*., 1996). It is particularly effective at high humidity (> 98% r.h.). However, at reduced water availability, particularly in the field, establishment of the antagonist and biocontrol is more difficult to achieve. Water activity (a_w) is a term which accurately describes the actual availability of water for microbial growth and is the ratio of water in a substrate to that in pure water at a standard temperature and pressure (Magan & Lacey, 1988). Thus we were interested in mechanisms which could improve the environmental competence of this biocontrol agent to allow effective establishment and survival under fluctuating field conditions and thus improve its biocontrol potential. Physiological methods for improving tolerance to such environmental stresses are considered to be fundamental to enable the development of effective and consistent microbial biocontrol agents (Deacon, 1991).

Low and high mol. wt polyhydroxy alcohols (polyols) such as glycerol, erythritol, and arabitol and mannitol, are often accumulated in fungal cells under low a_w (Beever & Laracy, 1986; Hocking, 1986; Ellis, Grindle & Lewis, 1991; Kelly & Budd, 1991; Van Eck, Prior & Brandt, 1993). Intracellular accumulation of these polyols reduces cytoplasmic a_w, but does not disrupt enzyme structure and function, thus allowing metabolic activity to continue during periods of water stress (Brown, 1978). Recent ecophysiological studies on ento-

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mogenous biocontrol fungi have demonstrated that it is possible to physiologically manipulate growth conditions, carbon sources and carbon:nitrogen ratios to channel specific low mol. wt polyols such as glycerol and erythritol into mycelium and propagules of filamentous fungi, resulting in improved and more rapid germination under water stress conditions (Hallsworth & Magan, 1994*a*; Hallsworth & Magan, 1995; Hallsworth & Magan, 1996). Conidia modified in this way may be more pathogenic to target pests at low relative humidity than those containing traces of these low mol. wt polyols (Hallsworth & Magan, 1994*b*, *c*). Initial studies with physiologically manipulated inocula of a biocontrol agent, *Epicoccum nigrum*, containing high concentrations of glycerol and erythritol have also been found to give better control of brown rot (*Monilinia laxa*) of peaches than unmodified inocula (Pascual, Magan & Melgarejo, 1996).

The disaccharide trehalose protects membrane and protein structure during dehydration and upon rehydration (Crowe, Crowe & Chapman, 1984; Carpenter & Crowe, 1988; Colaco *et al*., 1992; Leslie *et al*., 1994). It has been suggested that trehalose enhances desiccation tolerance of conidia of the biocontrol fungi *Trichoderma harzianum* (Harman *et al*., 1991), and *Aspergillus japonicus* (Gornova *et al*., 1992). Trehalose concentrations of greater than 10% have been found to be critical for stress resistance to freezing and freeze-drying of the industrial yeast *Saccharomyces cerevisiae* (Van Dijck *et al*., 1995). Hallsworth & Magan (1995) showed, however, that elevated trehalose concentrations in conidia of the filamentous fungi *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces farinosus* did not improve stress tolerance at lowered water availability, although they did prolong shelf-life.

This study was carried out (*a*) to determine the effect of modifying nutrient substrates and their concentrations, and a_w on the intracellular accumulation of polyols (glycerol, erythritol, arabitol and mannitol) and sugars (glucose and trehalose) in cells of *Candida sake*; (*b*) to evaluate the relationship between physiologically modified *C. sake* inocula and improved stress tolerance competence; and (*c*) to determine the conservation of biocontrol efficacy of such modified inocula against the pathogen *Penicillium expansum* Link.

MATERIALS AND METHODS

Organisms and media

The isolate used in this study was *Candida sake* (strain CPA-1) from UdL-IRTA (Lleida, Spain). Stock cultures were stored at 5 °C and had been sub-cultured on nutrient yeast dextrose agar (NYDA). A nutrient yeast dextrose broth (NYDB; nutrient broth, 8 g l^{-1}; yeast extract 5 g l^{-1}; dextrose 10 g l^{-1}; 0·995 water activity (a_w)) was used as the basal medium. It was modified by the addition of glucose (398 g l^{-1}) or glycerol (184 g l^{-1}) to obtain 0·96 a_w and trehalose (95 g l^{-1}) to obtain 0·97 a_w. The basic medium was also diluted 1:4 (NYDB25, 25% strength) and modified as above to 0·96 and 0·97 a_w, respectively. The a_w of the media was determined with a Novasina Humidat IC II dew point meter (Novasina AG, Zurich, Switzerland).

In all experiments 100 ml of media in 250 ml Erlenmeyer flasks for each treatment were inoculated with a known concentration of *C. sake* (10^4 cfu ml^{-1}) and were cultured with agitation on a rotary shaker (150 rpm) at 25°. After 24 and 48 h of incubation three replicates of each treatment were destructively sampled to obtain yeast cells for quantifying polyols and sugar concentrations, and for comparison of a_w stress tolerance of treatments. All experiments were carried out in triplicate and repeated.

Evaluation of viability and growth of physiologically characterized C. sake *in unstressed and stressed media*

Two separate experiments were carried out to determine the environmental competence of modified *C. sake* cells to grow under various a_w stress conditions. In the first experiment *C. sake* cells grown in different media were removed after 24 and 48 h and spread-plated on to the surface of 9 cm Petri plates containing NYDA modified with polyethylene glycol (PEG 200/300; 1·25 M and 1·0 M respectively) to 0·935 a_w. Plates of the same a_w were sealed in polyethylene bags to prevent water loss and incubated at 25°. The viability of yeast cells was counted after 48 h.

The second experiment compared the viability and growth of *C. sake* obtained from both unstressed (0·995 a_w) and stressed (0·95 a_w) NYDA-based media. Using the concentrations detailed above, the antagonist was inoculated into 250 ml Erlenmeyer flasks containing: NYDB, NYDB50 (diluted to 50%) or NYDB25 (diluted by 75%) and supplemented with glycerol or glucose to 0·96 a_w as described previously. All treatments were carried out in triplicate and were incubated with agitation (150 rpm) at 25°. After 24 and 48 h samples were spread-plated on unstressed (0·995 a_w) and stressed (0·95 a_w; 1·25 M PEG 200/0·5 M PEG 300) NYDA media and incubated at 25°. The number of cfu were determined after incubation for 24 and 48 h. All treatments were carried out in triplicate.

Extraction and detection of polyols and sugars

Suspensions of yeast cells of *C. sake* were placed in sterile 30 ml Universal bottles and centrifuged immediately for 15 min at 250 ***g*** (MSE Centaur 2). The yeast pellets were resuspended in HPLC grade water and centrifuged again to remove any residual liquid medium.

A known amount of fresh weight of *C. sake* cells (10–25 mg) was mixed with 1 ml HPLC grade water in a 2 ml Eppendorf tube and sonicated with a 4 mm sonicator probe for 2 min at an amplitude of 26 μm (Soniprep, Fisons). After immersion in a boiling water bath for 5 min the samples were left to cool and 0·67 ml acetonitrile was added to each sample to obtain the same ratio of acetonitrile:water as the mobile phase (40:60). The tubes were centrifuged for 10 min at 1150 ***g*** and the supernatant was filtered through 0·2 μm filters and injected in the HPLC for quantification of endogenous polyols and sugars.

Solutes were analysed and quantified by HPLC using a Hamilton HC-75 Ca^{2+} column and a Gilson RI Detector,

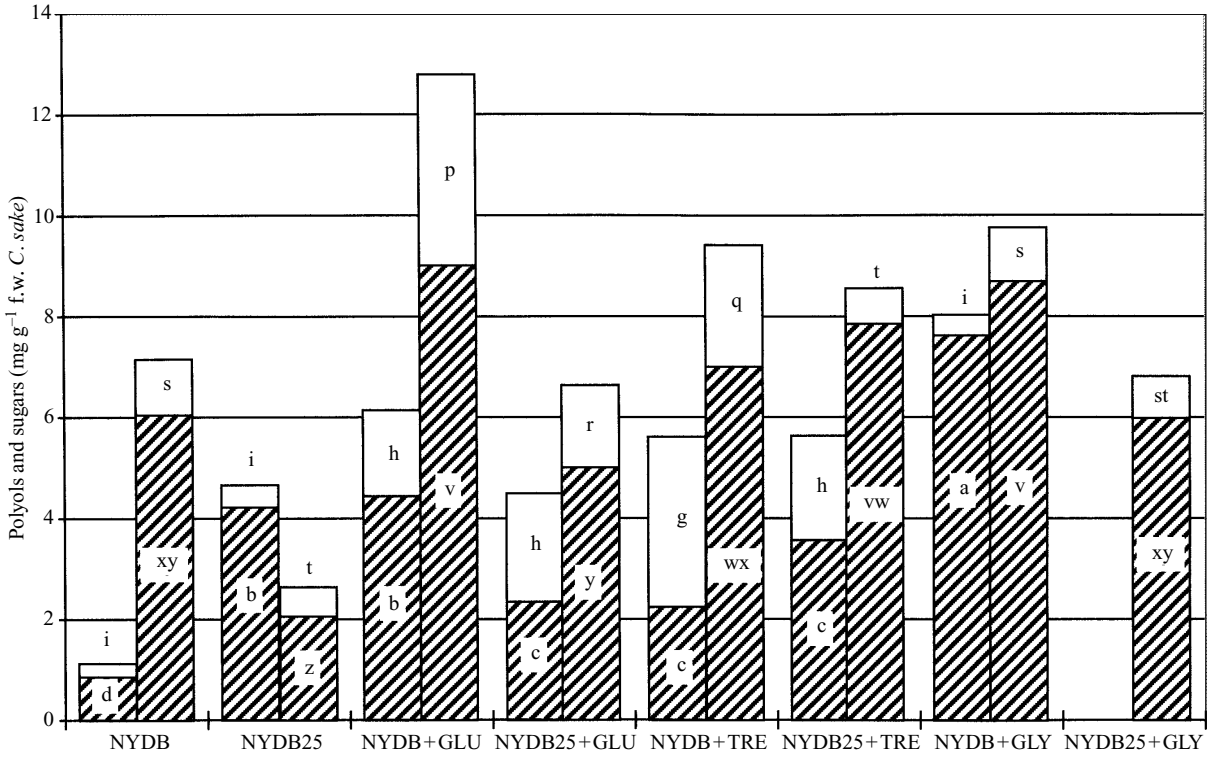

Fig. 1. Comparison of total intracellular polyols (▨) and sugars (□) (glucose and trehalose) present in cells of *Candida sake* grown on either NYDB, or NYDB modified with glucose, glycerol or trehalose. The separation of means was conducted according to Duncan's Multiple Range Test ($P < 0{\cdot}05$) and are shown for 24 h (first bar) and 48 h (second bar) incubation. Bars with different letters indicate significant differences between treatments.

specifically for sugar/polyol separation. The mobile phase used was acetonitrile : water (40 : 60). Six solutes were analysed : the polyols glycerol, erythritol, arabitol and mannitol and the sugars trehalose and glucose. This column allowed clear separation of the sugars sucrose, glucose, trehalose and fructose. In all cases three replicates of all treatments were analysed. The peak areas were integrated and compared with calibration curves constructed with standards of 50–600 ppm of each solute. Polyols, trehalose and glucose content were calculated as mg g^{-1} fresh weight of *C. sake* cells (Hallsworth & Magan, 1995).

Antagonistic activity of characterized inocula of C. sake *treatments against* P. expansum *in apples*

Suspensions of yeast cells of the different treatments above were grown in 250 ml flasks containing 100 ml of media with various a_w as described above and harvested after 24 and 48 h. The cells were centrifuged at 765 ***g*** for 10 min and cells were resuspended in 50 ml of water. The concentrations of the suspensions were adjusted to $7{\cdot}5 \times 10^5$ and $1{\cdot}6 \times 10^6$ cfu ml^{-1} according to a standard curve obtained spectrophotometrically by measuring transmittance at 420 mm (CECIL CE 1020) (Usall, 1995).

The apple cv. Golden Delicious used in this experiment was obtained from commercial orchards in Lleida, Catalonia, Spain and grown using standard cultural practices. Apples were surface-sterilized in alcohol, and wounded at the stem (top) and calyx (bottom) ends. The wounds were $3 \times 3 \times 3$ mm. A 25 µl suspension of the appropriate concentration of a suspension of *C. sake* (antagonist), from each treatment was applied to a wound, followed by inoculation with 20 µl of an aqueous suspension of *P. expansum* (1×10^4 cfu ml^{-1}). Three apples constituted a single replicate and each treatment was repeated three times.

Treated apples were incubated at 25° and 75 % r.h. for 7 d after which the diameter of decay were measured. The *P. expansum* strain had been isolated from decayed apples and shown to be highly pathogenic to apples (Usall, 1995).

Statistical treatment of the results

All results were analysed by an analysis of variance with SAS software (SAS Institute, version 6·03, Cary N.C.). Statistical significance was judged at the level $P < 0{\cdot}05$. When the analysis was statistically significant Duncan's Multiple Range Test was used for separation of the means. Pearson correlation coefficients between endogenous reserves and biocontrol effectivity or viability in a_w stressed media were calculated, respectively.

RESULTS

Nutrients and a_w *in relation to polyol, trehalose and glucose content of* C. sake *cells*

There were significant differences in total sugars and polyols in NYDB100 and NYDB25 with various additives depending on time of incubation and treatment (Fig. 1). Glucose and

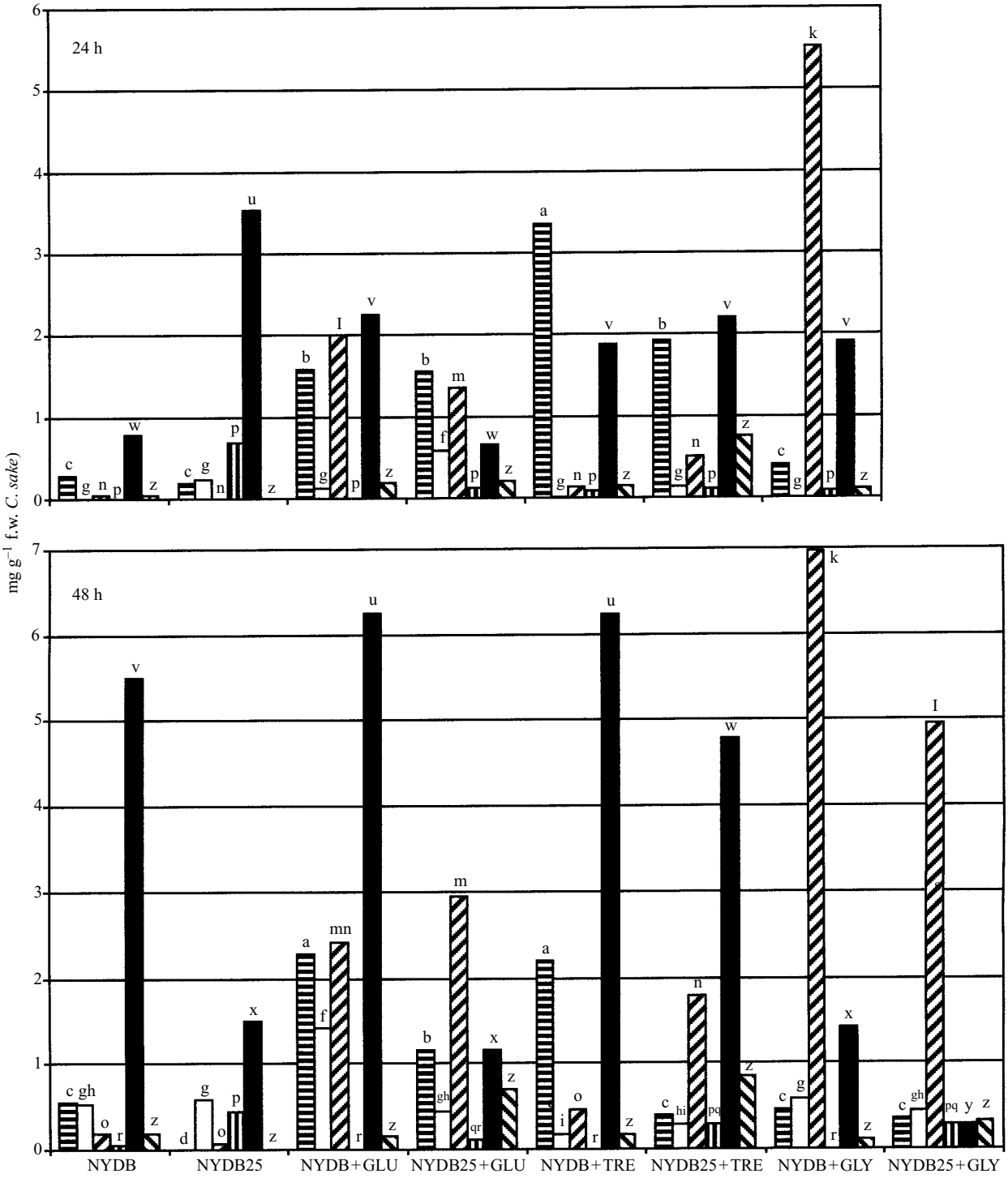

Fig. 2. Accumulation of intracellular sugars trehalose (▤), glucose (□), and the polyols glycerol (▨), erythritol (▥), arabitol (■) and mannitol (▧) in *Candida sake* grown on nutrient yeast dextrose based media (NYDB), either diluted to 25% strength (NYDB25), or modified with glucose (+GLU) or glycerol (+GLY), or trehalose (+TRE) to achieve 0·96 or 0·97 water activity (a_w) respectively. After 24 and 48 h at 25°. Results are means of three replicates per treatment. The separation of means are based on Duncan's Multiple Range Test for each endogenous reserve. Columns with different letters indicate significant differences ($P < 0·05$).

trehalose-modified media had the highest intracellular concentration of sugars present (up to 3·5 mg g^{-1} F.W.), the glycerol and unmodified treatments had the least.

Comparison of the total concentrations of polyols accumulated in the different treatments shows clearly that higher amounts were synthesized after 48 than 24 h incubation regardless of treatments. The highest concentrations (7–9 mg g^{-1} F.W.) were present in the richest medium (NYDB100) modified either with glucose or glycerol to 0·96 a_w.

The accumulation of polyols and sugars in *C. sake* cells in the NYDB medium, either diluted or modified with glucose or

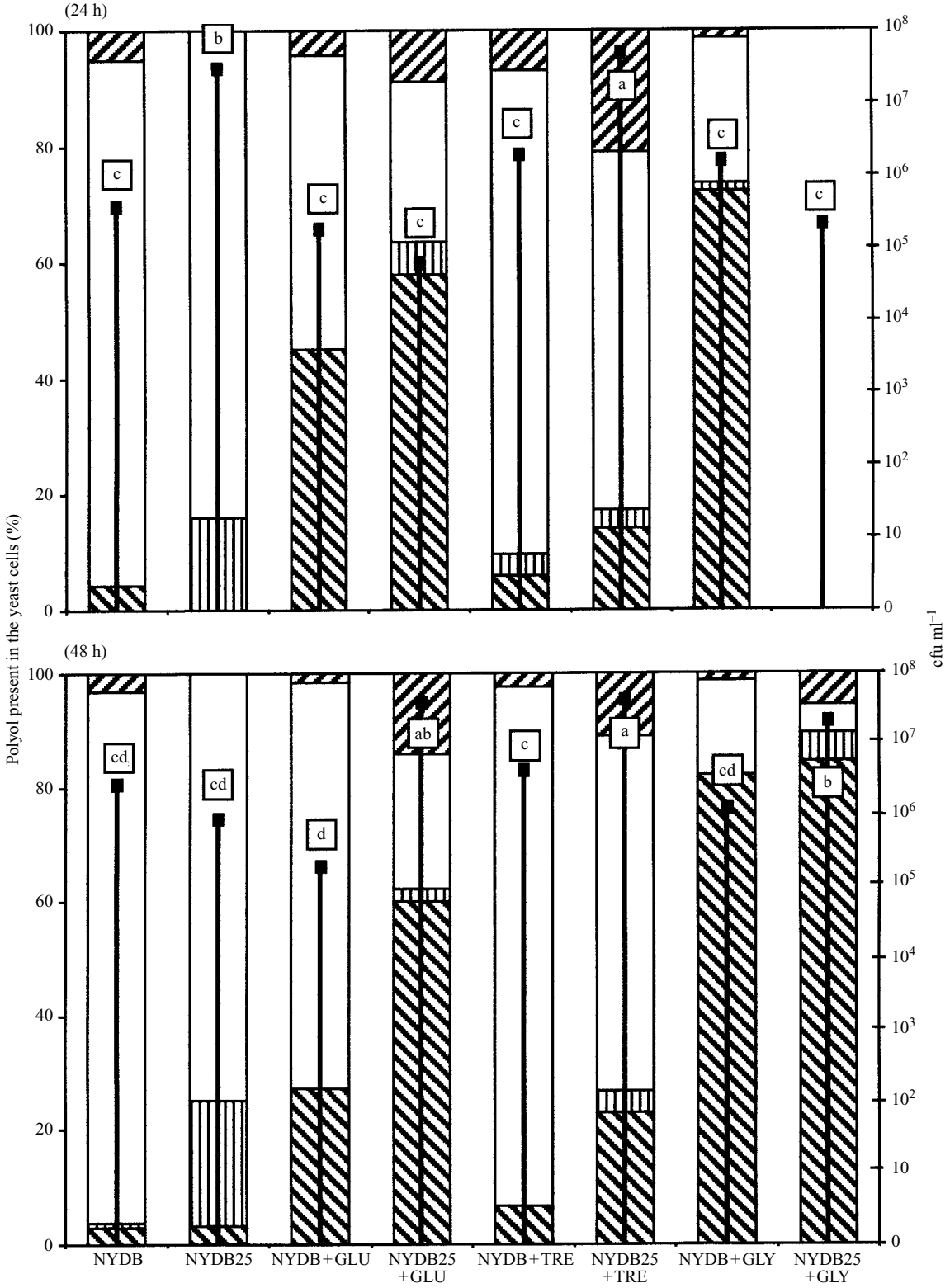

Fig. 3. Viability of *Candida sake* cells from different treatments at 0·935 a_w on NYDA modified with PEG. Colony forming units (cfu) ml^{-1} are superimposed on relative percentage of individual polyols present in the yeast cells after 24 and 48 h. Points with the same letter are not significantly different according to Duncan's Multiple Range Test ($P < 0·05$). Symbols: glycerol (▨), erythritol (▥), arabitol (□), mannitol (▨) and *C. sake* growth (■).

glycerol to 0·96 a_w or with trehalose to 0·97 a_w are shown after 24 and 48 h in Fig. 2. In normal unstressed NYDB medium, after 24 h, arabitol (< 1 mg g^{-1} F.W.) and trehalose ($< 0·4$ mg g^{-1}) were the major compounds accumulated. The concentrations of arabitol increased to > 5 mg g^{-1} after 48 h, accompanied by an increased glucose concentration (0·5 mg g^{-1}). Dilution of the medium concentration alone affected the accumulation of endogenous reserves, with

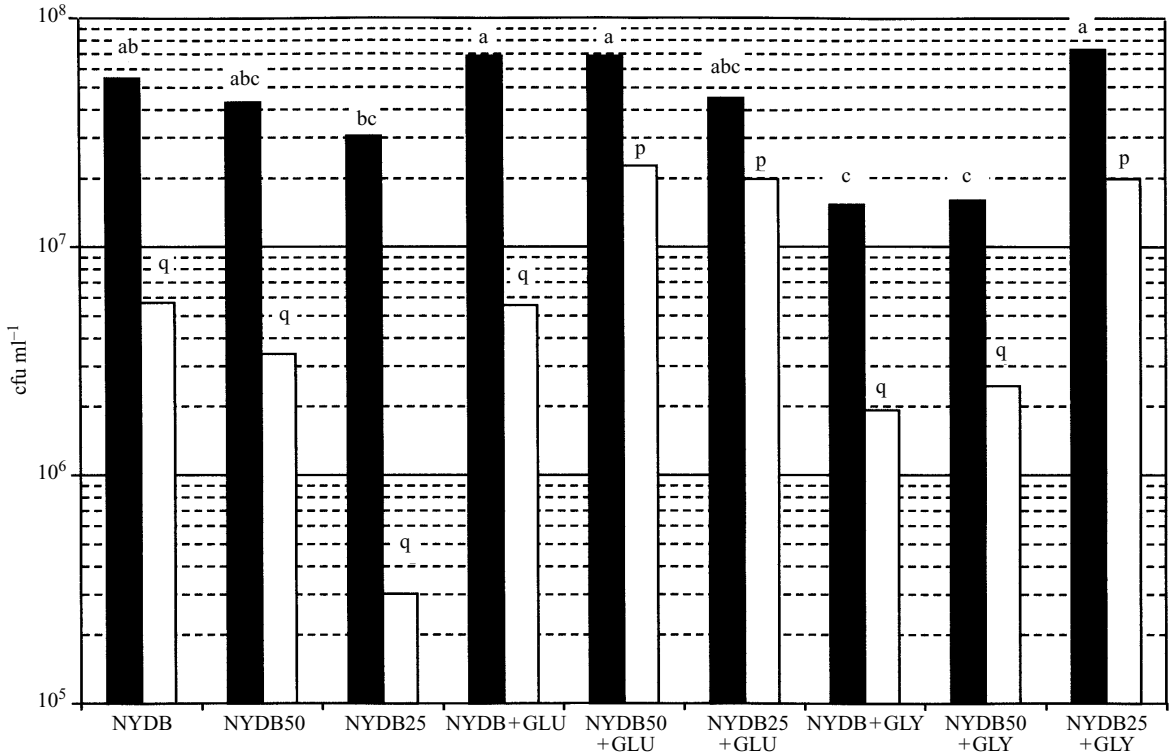

Fig. 4. Viability of *Candida sake* cells of each treatment in unstressed (0·995 a_w, ■) and stressed (0·95 a_w, □) media. The means for each medium are separated according to Duncan's Multiple Range Test ($P < 0·05$).

arabitol (1·5 mg g^{-1}) and erythritol as the major polyols, and glucose as the main sugar at 48 h. Either modifying the a_w alone, or use of diluted medium + reduced a_w resulted in significant changes in the accumulation patterns of polyols and sugars. For example, glycerol content of cells increased significantly, especially in the glycerol-modified media (5–7 mg g^{-1} F.W.) with a significant reduction in arabitol content (1·25 mg g^{-1}), and some of glucose and trehalose. No data are, however, presented for the NYDB25 + glycerol treatment after 24 h because of poor growth. In contrast, in the normal strength NYDB100 + glucose treatment arabitol was still the major polyol (> 6 mg g^{-1}) after 48 h, but glycerol, trehalose and glucose increased significantly. Dilution of the medium and modification of the a_w resulted in the greatest accumulation of glycerol, arabitol and trehalose. In normal strength medium and plus trehalose, treatments arabitol (4·5–6 mg g^{-1}) and trehalose were the major compounds accumulated by the yeast cells. In most cases in the weaker medium (NYDB25) trehalose content decreased after 48 h of incubation in comparison to that present after 24 h. Erythritol and mannitol contents were always low (< 0·9 mg g^{-1} F.W.) in cells of *C. sake*.

Stress tolerance of C. sake *cells in low* a_w *media*

In the first experiment, the cells from each treatment were plated after both 24 and 48 h onto 0·935 a_w medium modified with PEG200/300 to examine tolerance of low a_w. There were significant differences from those grown on unmodified media with respect to the viability of the cells of the *C. sake* treatments after both 24 and 48 h (Fig. 3). When data was superimposed on the percentages of polyols in each treatment for comparison, it was evident that larger populations were recovered on the 0·935 a_w medium from the treatments NYDB25 + glucose, NYDB25 + trehalose and NYDB25 + glycerol at 48 h than from unmodified controls.

There was a significant correlation between viability in stressed media and intracellular concentrations of arabitol (Pearson coefficient = −0·51, $P < 0·05$) and mannitol (Pearson coefficient = 0·84, $P < 0·01$). No correlation with other polyols or sugars was found.

In a second experiment the study was expanded to evaluate the capability of a wider range of treatments for viability on both normal unstressed (0·995 a_w) and on stressed (0·95 a_w) media. In this experiment the medium was diluted to 50 or 25% of the normal strength and again modified with glucose or glycerol. *C. sake* cells from NYDB50 + glucose, NYDB25 + glucose and NYDB25 + glycerol treatments grew better on the 0·95 a_w medium after 48 h of incubation, than unmodified control cells (Fig. 4). Interestingly, significant improvements in viability were also observed on unstressed media compared to unmodified control cells.

Biocontrol and antagonistic activity of modified C. sake *treatments against* P. expansum *on apples*

All *C. sake* treatments (NYDB, diluted and modified a_w) strongly improved inhibition of the development of *P. expansum* rot (Table 1). Rot reduction was in all cases greater than 60%, and cells grown on 50% and 25% diluted NYDB

Table 1. Biocontrol efficacy of *Candida sake* (% rot reduction) grown in different modified and unmodified media against *Penicillium expansum* rot of apples. Fruits were wounded, inoculated with the antagonist and challenged with 1×10^4 cfu ml^{-1} of *P. expansum*, and incubated for 7 d at 25°. The separation of means was conducted according to Duncan's Multiple Range Test ($P < 0{\cdot}05$). Treatments in each column with different letters are significantly different

	C. sake concentration (cfu ml^{-1})	
	$7{\cdot}5 \times 10^5$	$1{\cdot}6 \times 10^6$
NYDB	80·2 d	76·4 e
NYDB50	81·8 cd	84·8 c
NYDB25	72·8 f	82·0 cd
NYDB + GLU	82·9 cd	91·9 b
NYDB50 + GLU	92·2 b	96·7 a
NYDB25 + GLU	82·9 cd	95·8 a
NYDB + GLY	68·8 g	64·0 h
NYDB50 + GLY	72·7 f	69·5 g
NYDB25 + GLY	90·5 b	80·9 d

medium amended with glucose to 0·96 a_w with modified endogenous reserves gave significantly increased control in the range of 80–96%.

Glycerol treatments led to better biocontrol efficacy at modified strength than unmodified ones, with medium diluted to 25% presenting the best results in relation to other glycerol treatments.

Significant correlations of rot reduction with both intracellular concentrations of trehalose (Pearson coefficient = 0·58, $P < 0{\cdot}05$), and with total sugars (Pearson coefficient = 0·52, $P < 0{\cdot}05$) were found. For other polyols and sugars there was no correlation.

DISCUSSION

This study has shown that manipulation of the growth of the biocontrol yeast *C. sake*, by changing either nutrient concentration alone or by modifying both nutrient status and water stress, significantly affected intracellular accumulation of both individual and total polyols and glucose and trehalose. It had previously been found that increasing the carbon concentration not only changes the C:N ratio, but also reduces a_w markedly (Hallsworth & Magan, 1994*b*). The significant increase in total polyols and sugars demonstrated that under certain nutrient/water stress conditions greater amounts of these endogenous reserves are accumulated than in rich media commonly used for their cultivation. When considering the function of polyols in intracellular osmotic adjustment, however, the roles of individual polyols become important as they are differentially effective as compatible solutes. High mol. wt polyols (e.g. mannitol) cause slight inhibition of enzyme activity compared to low mol. wt polyols (e.g. glycerol) at equivalent concentrations (Chirife, Favetto & Ferro Fontan, 1984). Thus it was interesting to discover that yeast cells with a particular mixture of intracellular polyols/sugars are more tolerant of lowered a_w than others. For example, yeast cells with a mixture of predominantly glycerol, arabitol, and trehalose and glucose, and with very much lower concentrations of mannitol and erythritol, were significantly more tolerant of low water availability than unmodified yeast cells predominantly containing intracellular arabitol.

Previously, in rich media with *Candida albicans* (Pfyffer & Rast, 1988), and in low a_w media with *C. cacaoi* and *C. magnoliae* (Van Eck *et al.*, 1993), it was demonstrated that high mol. wt arabitol and the low mol. wt glycerol were the major polyols accumulated intracellularly. In the latter study a_w was only modified with glucose and NaCl. Work with *Dendryphiella salina* and *Neurospora crasssa* has suggested that the total soluble carbohydrates in fungi in relation to water stress remain constant (Ellis *et al.*, 1991; Jennings, 1995). With *N. crassa* grown in glucose media modified with NaCl, the concentrations of glycerol to mannitol was at a ratio of about 9:1. With fructose and the same water stress, however, the ratio of the two polyols was about 2:1. In the present study different patterns were observed with significant ($P = 0{\cdot}05$) increases in both total, and in some individual polyols, especially when media were modified with glucose or glycerol to 0·96 a_w as well as in combination with medium dilution.

We have paid attention specifically to intracellular accumulation of polyols and sugars because of the interest in finding ways for conservation of quality and effective viability of the biocontrol agent. Previously, no attempt has been made to evaluate the effect that such manipulation of the fungal agent's physiology has for improving tolerance of water stress and the potential for ecological competence in the environment. This is the first detailed study to demonstrate that physiologically modified and characterized yeast cells with known concentrations of polyols and sugars can improve viability of the cells under low water stress conditions. Previously, sclerotia with modified polyols were found to germinate faster than unmodified sclerotia, but this was only tested in a normal unstressed medium (Al Hamdani & Cooke, 1987). Significantly improved germination was demonstrated for conidia of some entomogenous fungi at reduced a_w ($< 0{\cdot}90$), when they contained elevated concentrations of glycerol and erythritol (Hallsworth & Magan, 1995). It is important to note that the accumulations of polyols and sugars observed in our study in relation to combined manipulations of nutrient status and a_w are significantly greater than those recently found in propagules of entomogenous biocontrol fungi following manipulation of pH and temperature (Hallsworth & Magan, 1996).

In our experiments, we found that culture age also can have an impact on accumulation of endogenous reserves, since in some treatments after 48 h there was a decrease in sugars relative to that present after 24 h. Thus time of incubation can also have a profound effect on the final quality and viability of the biocontrol agent and this may differ from species to species. In yeasts, depending on the solute used, polyols have been found to be actively released from yeast cells depending on the amount of water stress and the culture age (Blomberg & Adler, 1992; Jennings, 1995). In propagules of filamentous fungi polyols may be slowly metabolized and ultimately used in respiration or converted to higher mol. wt compounds such as glycogen. They can also be transported from the conidia to the mycelium or diffuse into the conidiophore wall (Kiyosawa, 1991).

More attention has been paid to improving desiccation tolerance by increasing the concentrations of sugars, particularly trehalose, in *Saccharomyces cerevisiae* (Van Dijck *et al*., 1995). By manipulating growth conditions this yeast increased intracellular concentrations of trehalose (on a dry weight basis) to more than 10%, which is the threshold above which stress resistance to freezing and freeze drying is optimum. Trehalose can enhance the resistance of cellular components against adverse conditions such as extreme temperatures, dehydration, or osmotic stress (Van Laere, 1989; Miekle *et al*., 1991; Piper, 1993). Effects on polyols were not, however, considered in these studies.

The demonstration that following manipulation of intracellular concentrations of sugars and polyols, biocontrol potential can also be conserved is of particular significance. This suggests that significant ecophysiological changes in the modified yeast cells do not affect biocontrol potential, and indeed may improve control. This indicates that modified stress-tolerant inocula of such yeasts may survive and become more effectively established on the surfaces of fruit in naturally fluctuating environmental conditions and that this could give the biocontrol agent a competitive advantage enabling more effective preemptive exclusion of pathogens such as *P. expansum* from such niches. This could provide a method for improving consistency and efficacy of such biocontrol agents in the field. The findings reported here demonstrate that ecophysiological manipulation of such yeasts has the potential for significantly improving the quality of such biocontrol agents by improving stress tolerance, and perhaps field performance and biocontrol.

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